LAW OFFICES
SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 PENNSYLVANIA AVENUE, N.W.
WASHINGTON, D.C. 20037-3202
TELEPHONE (202) 293-7060
FACSIMILE (202) 293-7860

CALIFORNIA OFFICE
1010 EL CAMINO REAL
MENLO PARK, CA 94025
TELEPHONE (650) 325-5800
FACSIMILE (650) 325-6606

JAPAN OFFICE
TOEI NISHI SHIMBASHI BLDG. 4F
13-5 NISHI SHIMBASHI 1-CHOME
MINATO-KU, TOKYO 105, JAPAN
TELEPHONE (03) 3503-3760
FACSIMILE (03) 3503-3756

February 8, 2000

ATTN: BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

ATTN: BOX PCT

Re: Application of **Shigeto UCHIYAMA, Tomomi UENO, Megumi KUMEMURA, Kiyoko IMAIZUMI, Masaki KYOSUKE and Seiichi SHIMIZU**
ISOFLAVONE-CONTAINING COMPOSITION
Our Reference: Q57711
PCT/JP98/03460, filed August 4, 1998

Dear Sir:

Applicants herewith submit the attached papers for the purpose of entering the National stage under 35 U.S.C. § 371 and in accordance with Chapter II of the Patent Cooperation Treaty. Attached hereto is the application identified above which is a verified translation **of PCT International Application No. PCT/JP98/03460, filed August 4, 1998,** comprising the specification, claims, executed Declaration and Power of Attorney, three (3) Receipts of Deposit from Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (FERM BP-6435, 6436 and 6437), International Preliminary Examination Report, International Search Report with PTO Form 1449, executed Assignment and PTO Form 1595.

The Office's attention is directed to the signature of the fourth inventor on the attached executed documents. The fourth inventor correctly signed his name according to the name order used in his country with the family name being set forth first. Accordingly, the typed name of the fourth inventor and the signature of the fourth inventor do correspond with each other.

The Government filing fee is calculated as follows:

Total Claims	32 - 20 =	12 x $18 =	$ 216.00
Independent Claims	6 - 3 =	3 x $78 =	$ 234.00
Base Filing Fee	($840.00)		$ 840.00
Multiple Dep. Claim Fee	($260.00)		$ 000.00
TOTAL FILING FEE			**$ 1,290.00**
Recordation of Assignment Fee			$ 40.00
TOTAL U.S. GOVERNMENT FEE			**$ 1,330.00**

Checks for the statutory filing fee of **$ 1,290.00** and Assignment recordation fee of **$ 40.00** are attached. You are also directed and authorized to charge or credit any difference or overpayment to Deposit Account No. 19-4880. The Commissioner is hereby authorized to charge any fees under 37 C.F.R. 1.492; 1.16 and 1.17 which may be required during the entire pendency of the application to Deposit Account No. 19-4880. A duplicate copy of this transmittal letter is attached.

Priority is claimed from:

Japanese Patent Application	Filing Date
214604/1997	August 8, 1997

The Office is invited to contact the above firm on any question which might arise on the above-named application. Any contact that the Office might need to make should be directed to the undersigned at (202)293-7060.

Respectfully submitted,
SUGHRUE, MION, ZINN, MACPEAK & SEAS
Attorneys for Applicant(s)

By _____
Waddell A. Biggart
Registration No. 24,861

WAB:clf

ISOFLAVONE-CONTAINING COMPOSITION

TECHNICAL FIELD

The present invention relates to an isoflavone-containing composition and more particularly to a novel composition either comprising a daidzein-containing substance and a strain of microorganism capable of metabolizing daidzein to equol or comprising equol obtained by causing said strain of microorganism to act upon said daidzein-containing substance, which composition is useful for the prevention and alleviation of unidentified clinical syndrome and conditions associated with the menopause in middle-aged to elderly women.

BACKGROUND ART

The documented collaborative research of National Cancer Center of Japan and Helsinki University (Finland) attributes the low incidence of gender-specific neoplastic diseases such as carcinoma of the prostate in men and carcinoma of the breast or ovary in women among the Japanese as compared with the European and American people to the greater intake by the Japanese of soybean-derived foods containing various isoflavonoids and the consequent well-coordinated balance of hormones (H. Adlercreutz, et al., (1992) Lancet, 339, 1233; H. Adlercreutz, et al., (1992) Lancet, 342, 1209-1210).

Recently, there has been a mounting interest in the

fact that isoflavonoids have estrogen (female hormone)-like activity (A. Molteni, et al., (1995) J. Nutr., 125, 751S-756S), and it has been reported that these compounds are effective in osteoporosis which develops after the menopause when estrogen secretions have subsided or ceased (D. Agnusdei, et al., (1995) Bone and Mineral, 19 (Supple), S43-S48) as well as in menopausal syndrome (D. D. Baird, et al., (1995) J. Clin. Endocrinol, Metab., 80, 1685-1690; A. L. Murkies, et al., (1995) Maturitas., 21, 195-198).

According to the result of a survey undertaken by Margaret Lock (M. Lock, et al., (1988) Maturitas ., 10, 317-332), the incidence of menopausal syndrome among the Japanese women is extremely low as compared with the Canadian counterparts. Based on the report, H. Adlercreutz and coworkers conjecture that the Japanese women ingest large amounts of processed soybean foods such as tofu, miso, soy sauce, etc. and, hence, the plant estrogens (isoflavonoids) occurring in those foods are responsible for the low incidence of menopausal syndrome. Comparing the urinary excretions (24-hour urine) which are known to reflect the amount of absorption of isoflavonoids actually ingested, the same authors further report that compared with the Western women, the urinary excretions in the Japanese women are tens of times as high (C. Herman,

et al., (1995), J. Nutr., 125, 757S-770S).

It is, thus, considered that the intake of isoflavonoids such as daidzein, genistein, daidzin, genistin, etc. is effective for the alleviation and prevention of postmenopausal osteoporosis and menopausal syndrome. Particularly, the postmenopausal life expectancy in women has reportedly increased to more than 30 years owing to the recent trend toward longevity and the alleviation and prevention of various diseases and symptoms which may develop after the menopause have important meanings in that they would lead to improvements in quality of life (QOL).

However, the above report, i.e. the survey report on the amount of intake of isoflavonoids and the urinary excretions of isoflavonoids in the middle-aged to elderly women in Japan reflects the results generated in a limited rural area and no substantive information is available. Moreover, the correlation between the frequency of menopausal syndrome and the amount of intake of isoflavonoids has not been squarely analyzed and revealed.

Therefore, the object of the present invention is to provide a novel composition which is effective for the prevention and alleviation of the so-called unidentified clinical syndrome in middle-aged to elderly women, inclusive of menopausal syndrome, for which no effective

means of prevention or alleviation has been available.

To accomplish the above object, the inventors first conducted a dietary survey, determination of urinary excretions of isoflavonoids, and a questionnaire survey about menopausal syndrome (unidentified clinical syndrome) in perimenopausal women in a broad geographical area including urban communities.

According to the results of the above investigation conducted in 116 women aged between 40 and 60 who belonged to Fukuoka Dietitian Association, the average amounts of intake of isoflavonoids were 9 mg/day for daidzein and 13 mg/day for genistein. The average urinary excretions of isoflavonoids were 19.6 μmol/day for daidzein and 10.0 μmol/day for genistein, and the average excretions of equol, a metabolite of daidzein, was 11.9 μmol/day (mean of subjects in whom it was detected). Incidentally, although daidzein and genistein were detected in all the subjects, equol was detected only in 46 (51.6%) of the 95 subjects.

Furthermore, women with paramenia and those within 5 years of the menopause being taken together as menopausal subjects, a questionnaire survey was conducted using 17 items which are in routine use in the diagnosis of menopausal syndrome [17 items as a modification of Kupperman menopausal index (Kupperman H. S., et al., (1953),

J. Clin. Endocrinol. Metabol., 13, 688-703), i.e. 1. hot flushes, 2. perspiration, 3. local sensation of cold, 4. shortness of breath, 5. numbness of limbs, 6. hypesthesia, 7. difficulty in falling asleep, 8. fitful sleep, 9. irritability, 10. nervousness, 11. melancholy, 12. vertigo, nausea, 13. weakness(fatigue), 14. stiff shoulders, pain in joints, muscular pain, 15. headache, 16. palpitation, 17. tingling sensation] and the simplified menopausal index (SMI) was calculated. With subjects with SMI values not less than 20 being taken as a group of high climacteric symptoms and those with SMI values not greater than 19 as a group of low climacteric symptoms, the amount of intake of isoflavonoids and the urinary excretion of isoflavonoids were respectively compared between the groups.

As a result, whereas no intergroup difference was found in the amount of intake of daidzein, the amount of intake of genistein tended to be lower in the group of high climacteric symptoms at $p = 0.0643$. With regard to the urinary excretions of isoflavonoids, no intergroup difference was found whether for daidzein or for genistein but the excretions of equal were significantly low ($p<0.01$) in the group of high climacteric symptoms.

From the above results, the inventors found that unidentified clinical symptoms in menopausal women are

more closely related to the amount of intake of genistein and the urinary excretion of equal, among various isoflavonoids.

In the past the relationship of the amounts of intake and urinary excretion of isoflavonoids as a whole to their physiological effect has been discussed without regard to specific kinds of isoflavonoids such as daidzein and genistein but the results of the survey conducted by the inventors in the Japanese middle-aged and elderly women made it clear that not only the amounts of intake and urinary excretion of isoflavonoids in general but also the amount of intake of genistein and the urinary excretion of equol, particularly the rate of metabolic conversion of daidzein to equol, are closely related to unidentified clinical climacteric symptoms in menopausal women.

In another study undertaken by the inventors in healthy adult volunteers (25 - 33 years of age), it was found that the urinary excretions of isoflavonoids (daidzein and genistein) after single ingestion of soy milk, a representative isoflavonoid-containing food, are increased in a dose-related fashion but in subjects who showed no urinary excretion of equol, equol was not detected in the urine even when the amount of intake of soy milk was increased two-fold, indicating the existence of individual difference in the metabolic pathway from

daidzein to equol.

It is known that equol, which is a metabolite of daidzein, is not detected in isoflavonoid-containing foods such as processed soybean products nor is it taken into the body from foods in ordinary diets (K. Reinli, et al., (1996), Nutr. Cancer, 26, 123-148).

Based on the above findings the inventors did further research and, as a result, succeeded in the development of a novel composition which comprises a strain of microorganism having the ability (metabolic activity) to elaborate equol from daidzein and either daidzein or a suitable substance containing daidzein in combination, and a novel composition which comprises equol obtained by causing said strain of microorganism to assimilate daidzein. The inventors then discovered that the intake of whichever of the above compositions is effective in the prevention and alleviation of unidentified clinical syndrome in middle-aged and older women and have accordingly developed the instant invention.

DISCLOSURE OF INVENTION

The present invention in a first aspect provides a composition, in the form of a food or a pharmaceutical product, which comprises a daidzein-containing substance and a strain of microorganism capable of metabolizing daidzein to equol as essential ingredients (which

composition will hereinafter be referred to as "isoflavone-containing composition").

The present invention in a second aspect provides a composition, in the form of a food or a pharmaceutical product, which comprises equol which is obtained by causing a strain of microorganism capable of metabolizing daidzein to equol to act upon a daidzein-containing substance (which composition will hereinafter be referred to as "equol-containing composition").

The present invention further provides said isoflavone-containing composition and equol-containing composition wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of Bacteroides ovatus, Streptococcus intermedius and Streptococcus constellatus and more particularly said isoflavone-containing composition and equol-containing composition wherein said strain of microorganism is at least one member selected from the group consisting of Bacteroides E-23-15, which has been deposited as FERM BP-6435, Streptococcus E-23-17, which has been deposited as FERM BP-6436, and Streptococcus A6G-225, which has been deposited as FERM BP-6437.

The present invention further provides said isoflavone-containing composition and equol-containing composition which further contain at least one ingredient

that favors the maintenance and growth of said strain of microorganism, for example at least one substance selected from the group consisting of galactosylsucrose, soybean-oligosaccharide, lactulose, lactitol and fructo-oligosaccharide.

The present invention further provides said isoflavone-containing composition and equol-containing composition wherein said daidzein-containing substance further contains at least one member selected from the group consisting of genistein, daidzin and genistin, more preferably soya isoflavone.

The present invention further provides said isoflavone-containing composition and equol-containing composition for the prevention and treatment of unidentified clinical syndrome in middle-aged to elderly women, inclusive of menopausal syndrome.

The present invention further provides said isoflavone-containing composition and equol-containing composition in the form of a food which is selected from the group consisting of drinks, dairy products, fermented milk, bars, granules, powders, capsules and tablets.

The present invention further provides said isoflavone-containing composition and equol-containing composition in the form of a pharmaceutical product which is selected from the group consisting of aqueous solutions,

emulsions, granules, powders, capsules and tablets.

The present invention in a further aspect provides a method for prevention and treatment of unidentified clinical syndrome or menopausal syndrome in middle-aged to elderly women which comprises administering an effective amount of said isoflavone-containing composition or equol-containing composition to a middle-aged or elderly woman in whom said prevention or treatment are needed.

The present invention further provides the use of a microorganism capable of utilizing a daidzein-containing substrate or daidzein to produce equol for the production of said isoflavone-containing composition and equol-containing composition which are effective for the prevention and treatment of unidentified clinical syndrome or menopausal syndrome in middle-aged to elderly women.

The present invention further provides a method of producing equol which comprises causing a strain of microorganism capable of metabolizing daidzein to equol to act upon daidzein.

The present invention further provides a strain of microorganism selected from the group consisting of Bacteroides E-23-15, which has been deposited as FERM BP-6435, Streptococcus E-23-17, which has been deposited as FERM BP-6436, and Streptococcus A6G-225, which has been

deposited as FERM BP-6437.

The isoflavone-containing composition of the invention is now described in detail.

In the isoflavone-containing composition of the invention, a daidzein-containing substance is used as one of its essential ingredients. This daidzein-containing substance includes not only daidzein as such but also daidzin which is a glycoside of daidzein and a variety of substances containing daidzein and/or daidzin. The daidzein present itself chiefly in soybean, kudzu and the like raw foods, their processed products such as tofu, aburage, soy milk, etc. and their fermentation products such as natto, soy sauce, miso, tempeh, etc. In the present invention, any of such raw foods, processed products and fermentation products can be used as said daidzein-containing substance. Particularly, the substances contain not only daidzein but also other isoflavonoids having estrogen-like activity, such as genistein, daidzin, genistin, etc., biochain A and formonetin which is a partially methylated precusor of genistein and daidzein, respectively, etc. and can be used with advantage for the purpose of the invention.

The daidzein-containing substance which is preferred for the practice of the present invention further includes soya isoflavone derived from soybeans, for

example commercial products such as "Fujiflavone (trade name) P10" from Fujicco, and isoflavonoids derived from plants such as red clove, alphalpha, etc.

In the isoflavone-containing composition of the invention, a strain of microorganism having an ability (metabolic activity) to produce equol from daidzein is used as the other essential ingredient. The microorganism includes those belonging to Bacteroides ovatus, Streptococcus intermedius, and Streptococcus constellatus. Particularly preferred among such microorganisms are Bacteroides E-23-15 (FERM BP-6435), Streptococcus E-23-17 (FERM BP-6436) and Streptococcus A6G-225 (FERM BP-6437), all of which were isolated from human stools and deposited for accession by the inventors.

The bacteriological characteristics of those strains of microorganisms are now described in detail.

(1) Bacteroides E-23-15, FERM BP-6435

I. Cultural characters

When cultured anaerobically using an anaerobic jar stuffed with steel wool at 37°C for 48 hours, this strain gives good to moderate growth on Eggerth-Gagnon (EG) agar, Blood Liver (BL) agar, or Gifu Anaerobic Medium (GAM). The colonies are circular, protuberant in a convex manner, with both the surface and peripheral edges being glabrous to slightly coarse. The colony color is grayish white on EG

agar or grayish brown on BL agar. Morphologically it is a gram-negative rod and shows polymorphism ranging from coccobacillus, single rod, elongated rod, etc., but the cells occur singly and not in chains. No sporogenesis is found.

II. Physiological characteristics

(1) Optimum temperature for growth: 37 °C

(2) Optimum pH for growth: 7.0

(3) Liquefaction of gelatin: +

(4) Hydrolysis of soluble starch: +

(5) Hydrolysis of esculin: +

(6) Indole production: -

(7) Urease: -

(8) Catalase: -

(9) Assimilation of carbon sources:

L-arabinose	+
D-xylose	+
D-glucose	+
Sucrose	+
L-Rhamnose	+
D-Raffinose	+
D-Mannitol	+
Indole	+
Lactose	+
Maltose	+

Salicin	+
Gelatin	+
Glycerin	+
D-cellobiose	+
D-mannose	+
D-melezitose	+
D-sorbitol	+
D-trehalose	+

(10) Organic acid composition after utilization of peptone or glucose:

Using PYF (peptone-yeast extract-Fildes) medium (containing about 5% of peptone), which is utilized in sugar fermentation test, and PYF medium supplemented with 0.5% final concentration of glucose, the strain was cultured anaerobically at 37°C for 72 hours and the organic acids in the resulting culture were assayed by HPLC. The results (unit: mM) are shown below.

Organic acid	PYF culture	Glucose-PYF Culture
Maleic acid	0.02	1.19
Succinic acid	0.01	3.20
Lactic acid	0.01	4.94
Formic acid	0.03	0.66
Acetic acid	0.29	2.62
Pyroglutamic acid	0.01	nd
Propionic acid	nd	nd
i-Butyric acid	1.71	0.23
n-Butyric acid	0.36	nd
i-Valeric acid	nd	0.19
n-Valeric acid	nd	nd

nd = not detected

The above morphological and biochemical characteristics, sugar fermentation test and organic acid production spectrum suggested that this strain was either of the gram-negative rods Bacteroides ovatus and Bacteroides uniformis but was decided to be a microorganism belonging to Bacteroides ovatus in view of its ability to utilize rhamnose. Accordingly this strain was named Bacteroides E-23-15 and deposited with National Institute of Bioscience and Human Technology (NIBH, Higashi 1-1-3, Tsukuba-shi, Ibaraki, Japan) as of July 7, 1997 under the accession number of FERM P-16312. This deposit was converted to a Budapest deposit on July 22, 1998 and assigned the accession number of FERM BP-6435.

(2) Streptococcus E-23-17 (FERM BP-6436)

I. Cultural characteristics

When cultured anaerobically in an anaerobic jar stuffed with steel wool at 37°C for 48 hours, this strain gives good to moderate growth on EG agar, BL agar or GAM. The colonies are circular and conical to protuberant in a centrally convex fashion, and have a ground glass-like to granular texture with a smooth or slightly coarse edge. The colonies on EG agar are transparent to grayish brown. Morphologically it is a gram-positive coccus, ellipsoidal or with slightly pointed ends. The cells occur singly or are diplococcal, forming irregular masses. No chain is formed. The strain is not sporogenic.

II. Physiological characteristics

(1) Optimum temperature for growth: 37 °C

(2) Optimum pH for growth: 7.0

(3) Liquefaction of gelatin: -

(4) Hydrolysis of soluble starch: -

(5) Hydrolysis of esculin: +

(6) Indole production: -

(7) Urease: -

(8) Catalase: -

(9) Assimilation of carbon sources:

L-arabinose	+
D-xylose	-
D-glucose	+
Sucrose	-

L-Rhamnose	+
D-Raffinose	-
D-Mannitol	+
Indole	-
Lactose	+
Maltose	+
Salicin	+
Gelatin	-
Glycerin	-
D-cellobiose	+
D-mannose	+
D-melezitose	-
D-sorbitol	±
D-trehalose	+

(10) Organic acid composition after utilization of peptone or glucose:

Using PYF (peptone-yeast extract-Fildes) medium (containing about 5% of peptone), which is utilized in sugar fermentation test, and PYF medium supplemented with 0.5% final concentration of glucose, the strain was cultured anaerobically at 37°C for 72 hours and the organic acids in the resulting culture were assayed by HPLC. The results (unit: mM) are shown below.

Organic acid	PYF culture	Glucose-PYF Culture
Maleic acid	0.04	Nd
Succinic acid	2.37	0.02
Lactic acid	0.02	nd
Formic acid	0.03	0.03
Acetic acid	3.32	0.07
Pyroglutamic acid	0.03	nd
Propionic acid	3.24	nd
i-Butyric acid	4.17	1.11
n-Butyric acid	nd	nd
i-Valeric acid	4.50	nd
n-Valeric acid	nd	nd

nd = not detected

The above morphological and biochemical characteristics, sugar fermentation test and organic acid production spectrum suggest that this strain belongs to either of the gram-positive cocci Luminococcus productus and Streptococcus constellatus but the strain differentiates itself from the type culture strain of Luminococcus productus in the ability to utilize sucrose, D-xylose and D-raffinose. Therefore, the inventors named the strain Streptococcus E-23-17 and deposited it with National Institute of Bioscience and Human Technology (NIBH, Higashi 1-1-3, Tsukuba-shi, Ibaraki, Japan) as of July 7, 1997 under the accession number of FERM P-16313. This deposit was subsequently converted to a Budapest deposit as of July 22, 1998 and assigned with the accession number of FERM BP-6436.

(3) Streptococcus A6G-225 (FERM BP-6437)

I. Cultural characteristics

When cultured anaerobically using an anaerobic jar stuffed with steel wool at 37°C for 48 hours, this strain shows good to moderate growth on EG agar, BL agar or GAM. The colonies are circular, conical to protuberant in a centrally convex fashion and have ground glass-like to granular texture with a smooth or slightly coarse peripheral edge. The colonies on EG agar are transparent to grayish white. Morphologically it is a gram-positive coccus, ellipsoidal or with slightly pointed ends. The cells occur singly or are diplococcal, forming irregular masses. No chain is formed. No sporogenesis is found, either.

II. Physiological characteristics

(1) Optimum temperature for growth: 37 °C

(2) Optimum pH for growth: 7.0

(3) Liquefaction of gelatin: -

(4) Hydrolysis of soluble starch: -

(5) Hydrolysis of esculin: +

(6) Indole production: -

(7) Urease: -

(8) Catalase: -

(9) Assimilation of carbon sources:

L-arabinose -

D-xylose	-
D-glucose	+
Sucrose	+
L-Rhamnose	-
D-Raffinose	+
D-Mannitol	-
Indole	-
Lactose	+
Maltose	+
Salicin	+
Gelatin	-
Glycerin	-
D-cellobiose	+
D-mannose	+
D-melezitose	-
D-sorbitol	-
D-trehalose	-

(10) Organic acid composition after utilization of peptone or glucose:

Using PYF (peptone-yeast extract-Fildes) medium (containing about 5% of peptone), which is utilized in sugar fermentation test, and PYF medium supplemented with 0.5% final concentration of glucose, the strain was cultured anaerobically at 37°C for 72 hours and the organic acids in the resulting culture were assayed by HPLC. The

results (unit: mM) are shown below.

Organic acid	PYF culture	Glucose-PYF culture
Maleic acid	nd	Nd
Succinic acid	0.21	0.03
Lactic acid	nd	35.36
Formic acid	0.55	1.66
Acetic acid	1.35	0.54
Pyroglutamic acid	nd	nd
Propionic acid	nd	nd
i-Butyric acid	2.04	nd
n-Butyric acid	nd	nd
i-Valeric acid	nd	nd
n-Valeric acid	nd	nd

nd = not detected

The above morphological and biochemical characteristics, sugar fermentation test and organic acid production spectrum suggest that this strain belongs to the gram-positive Streptococcus intermedius but the strain differentiates itself from the type culture strain of S. intermedius in the ability to utilize L-rhamnose and D-trehalose. Therefore, the inventors named the strain Streptococcus A6G-225 and deposited it with National Institute of Bioscience and Human Technology (NIBH, Higashi 1-1-3, Tsukuba-shi, Ibaraki, Japan) as of July 7, 1997, with the accession number of FERM P-16314 assigned. This deposit was subsequently converted to a Budapest deposit as of July 22, 1998 and assigned with the accession number of FERM BP-6437.

The above three strains of microorganisms isolated

by the inventors have the ability to utilize daidzein to elaborate equol as their most outstanding characteristic. The daidzein includes daidzein as the aglycone of an isoflavone glycoside such as daidzin. Daidzin is utilized by said microorganisms to give daidzein, and equol is then produced from this daidzein.

There has been no report on such a microorganism capable of producing equol. Therefore, the present invention further provides novel strains of microorganisms having the ability to produce equol.

The above strain of microorganism for use as an essential ingredient of the isoflavone-containing composition of the invention may generally be the live microorganism as such. However, it is not limited thereto but includes its culture, a crude or purified product thereof, and their lyophilyzates. Its proportion is not particularly restricted but can be judiciously selected according to the kind of microorganism, among other factors. For example, in the case of Streptococcus intermedius in fermented milk, the bacterial count is preferably controlled within the range of about 10^8 to 10^9 cells/ml. The bacterial count is determined by inoculating an agar medium with a diluted sample, incubating the inoculated medium anaerobically at 37 °C and counting the colonies formed. In the case of other strains of microorganisms,

too, the count determined in the above manner can be used as a rule of thumb.

The isoflavone-containing composition of the invention further preferably contains a nutrient component particularly suited to the maintenance and growth of the particular strain of microorganism. The nutrient component includes various oligosaccharides such as galactosylsucrose, soybean-oligosaccharide, lactulose, lactitol, fructo-oligosaccharide, and galacto-oligo-saccharide. The formulating amount of such nutrients is not particularly restricted but generally is preferably selected from the range of about 1 to 3 weight % based on the total composition of the invention.

The composition of the invention is generally prepared by blending predetermined amounts of said essential ingredients and other optional ingredients and processing the mixture into a suitable food form or pharmaceutical dosage form, such as drinks, dairy products, fermented milk, bars, granules, powders, capsules, tablets, etc. for food use or aqueous solutions, emulsions, granules, powders, capsules, tablets, etc. for pharmaceutical use. The production of such dosage forms can be carried out in the conventional manner. The carrier for use in the manufacture of such dosage forms includes edible carriers and pharmaceutically acceptable excipients and diluents.

Particularly in the case of a food form, a palatable and taste-improving carrier is preferred.

The particularly preferable examples of carrier include such masking agents as trehalose (manufactured by Hayashibara), cyclodextrin, Benekote BMI (manufactured by Kao Corporation), etc.

The blending ratio of said daidzein-containing substance, specific strain of microorganism and optional ingredients used for the maintenance and growth of the microorganism is not particularly critical. However, based on 100 g of the composition of the invention, the proportion of the daidzein-containing substance is preferably within the range of about 10-50 mg of daidzein contained therein. On the same basis, the proportion of the microorganism is preferably 10^9 to 10^{10} cells (as viable cells) and that of the oligosaccharide is preferably within the range of about 1-5 g.

Since the isoflavone-containing composition of the invention contains a strain of microorganism (primarily live cells) as mentioned above, the composition preferably should not be subjected to heating and/or pressurization in the course of processing into final products. Therefore, in processing the composition of the invention into such dosage forms as bars, granules, powders, tablets, etc., it is preferable to add the microorganism as

lyophilized cells as such or lyophilized cells coated with a suitable coating agent.

The composition of the invention may be optionally supplemented with various other food ingredients having nutritional values or various additives which are conventionally used in the manufacture of pharmaceutical products. The food ingredients mentioned above include calcium, vitamin B, vitamin D, vitamin C, vitamin E and vitamin K (particularly MK-7 (menaquinone-7) derived from Bacillus natto). Other examples of the substances that can be added include zinc and selenium.

The resulting isoflavone-containing composition of the invention is useful for the prevention and treatment of unidentified clinical syndrome, postmenopausal osteoporosis and other menopausal syndrome and symptoms in middle-aged and older women. Such prevention and treatment are achieved by administering or ingesting an effective amount of the above composition of the invention to a middle-aged or elderly woman who needs such prevention or treatment. The effective amount of the composition is not particularly restricted insofar as the prevention and treatment of unidentified clinical syndrome, postmenopausal osteoporosis or menopausal syndrome can be achieved with it. In general, the effective amount is preferably such that about 10-50 mg/day of daidzein and

at least about 10 mg/day of genistein can be taken.

The equol-containing composition of the invention is now described in detail.

The equol-containing composition of the invention comprises equol obtainable by causing a strain of microorganism capable of utilizing daidzein to produce equol to act upon a daidzein-containing substance.

The strain of microorganism may be the same as that of the above-described isoflavone-containing composition of the invention. The daidzein-containing substance on which said microorganism is caused to act can also be the same as that mentioned for the isoflavone-containing composition of the invention, thus including an isolated and purified form of daidzein, food materials containing it, processed matters or fermentation products thereof, soya isoflavone and isoflavones derived from kudzu, red clove, alphalpha, etc., products containing such isoflavones, for example tofu, soy milk, boiled soybeans, natto, soybean hypocotyl extract, etc.

The equol-containing composition of the invention is very safe because the active ingredient thereof is a native substance as mentioned above. Moreover, since it is prepared by using a microorganism, the composition is not only free from contamination with chemicals and the like contaminants but also advantageous in that it can be

obtained in high yield and at low production cost.

The equol-containing composition of the invention can be produced by conventional fermentation technology utilizing said daidzein-containing substance, preferably soya isoflavone or a food material containing it, as the substrate.

More particularly, the technology comprises sterilizing the substrate in solution form, adding the predetermined strain of microorganism thereto, and incubating the mixture at 37 °C either under anaerobic conditions or under aerobic stationary conditions for about 48-96 hours to let fermentation proceed. (Where necessary, a pH control agent, a reducing substance (e.g. yeast extract, vitamin K_1) can be added).

Taking Streptococcus intermedius as an example, the above cultural process can be more preferably carried out as follows. First, daidzein is dissolved in the range of 0.01-0.5 mg/ml in Modified GAM (Modified Gifu Anaerobic Medium) for culture of anaerobic bacteria. A seed culture prepared by growing the microorganism in Modified GAM for about 14 hours is then inoculated into the above daidzein-containing Modified GAM. The inoculum size may be 1/100 by volume of the medium. The incubated medium is incubated aerobically at 37 °C under stationary conditions for 48-96 hours.

The present invention further provides a method of producing equol utilizing such a strain of microorganism.

In the above fermentation system, there may be incorporated a nutrient which is particularly suited for the maintenance and growth of the microorganism. The nutrient includes oligosaccharides such as galactosyl-sucrose, soybean-oligosaccharide, lactulose, lactitol, fructo-oligosaccharide, and galacto-oligosaccharide. The amount of said nutrient is not particularly restricted but is preferably selected from the range of generally about 1-3 weight % based on the total composition of the invention.

The desired equol-containing culture broth can thus be obtained.

Isolation and purification of equol from the fermentation broth can be carried out in the conventional manner. A typical procedure may comprise adsorbing the fermentation broth on an ion exchange resin (e.g. DIAION HP20, Mitsubishi Kasei Corporation), eluting the objective substance with methanol, and concentrating the active fraction to dryness to provide crude equol.

The equol-containing composition of the invention can be produced, in a suitable food form or pharmaceutical dosage form, by formulating the equol-containing culture broth prepared as above or equol isolated therefrom with

other optional food materials.

The food form includes drinks, milk products, fermented milk, bars, granules, powders, capsules, and tablets. The pharmaceutical dosage form includes aqueous solutions, emulsions, granules, powders, capsules and tablets. Those food or pharmaceutical dosage forms can each be manufactured by the established technology. The carrier for use in the manufacture of such forms may be any of edible carriers and pharmaceutically acceptable excipients and diluents. Particularly in the case of foods, the carrier is preferably a palatable, taste-improving carrier.

The amount of equol in the resulting composition of the invention is not particularly restricted but can be determined according to the intended food form or pharmaceutical dosage form. Usually, however, based on 100 g of the total composition, the amount of equol is preferably about 10-50 mg.

The amount of intake of the composition of the invention is not particularly restricted but can be generally selected so that the urinary excretions of equol after ingestion of the composition will not be less than 5 μmole/day.

The equol-containing composition of the invention is useful for the prevention and treatment of unidentified

clinical syndrome in middle-aged to elderly women, typically symptoms of postmenopausal osteoporosis and menopausal syndrome.

BEST MODE FOR CARRYING OUT THE INVENTION

For a further detailed description of the invention, examples of preparation of the isoflavone-containing composition and equol-containing composition of the invention and an example of production of equol are presented below. It being to be understood, however, that the scope of the invention is not limited by those examples.

Example 1

Preparation of a drink

The ingredients according to the following recipe were weighed and blended to provide the composition of the invention in the form of a beverage.

Fermentation broth of water-soluble soybean protein	10 ml
Galactosylsucrose (55% content)	10.0 g
Vitamins & minerals	q.s.
Flavor	q.s.
Water	q.s.
Total	150 ml

The above fermentation broth of water-soluble soybean protein was prepared by dissolving 2.2 g of water-soluble soybean protein in 10 ml of water, adding

10^8 cells of Streptococcus A6G-225 (FERM BP-6437) thereto, and incubating the mixture at 37 °C for 48 hours.

Example 2

Preparation of a fermented milk

The ingredients according to the following recipe were weighed and blended to provide the isoflavone-containing composition of the invention in the form of fermented milk.

Water-soluble soybean protein	2.2 g
Galactosylsucrose (55% content)	10.0 g
Streptococcus A6G-225-fermented milk	100 ml
Vitamins & minerals	q.s.
Flavor	q.s.
Water	q.s.
Total	50 ml

The water-soluble soybean protein contained about 3-4% of daidzein (as analyzed by high-performance liquid chromatography; the same applies hereinafter). The Streptococcus A6G-225-fermented milk was prepared by adding 10^8 cells of Streptococcus A6G-225 (FERM BP-6437) to 1 liter of milk and incubating the mixture at 37°C for 24 hours.

Example 3

Preparation of a fermented soy milk lyophilizate

Using 1 ml of a suspension of about 10^7 cells/ml of

Streptococcus A6G-225 (FERM BP-6437), 100 g of soy milk was caused to undergo lactic acid fermentation at 37°C for 24 hours to provide equol. This product was lyophilized. The equol content of this freeze-dried powder was 0.1-0.3 weight %.

The above powder and other ingredients according to the following recipe were weighed and blended to provide the composition of the invention in the form of a fermented soy milk lyophilizate.

Fermented soy milk lyophilizate	2.2 g
Excipient	q.s.
Vitamins & minerals	q.s.
Flavor	q.s.
Total	20 g

As the excipient, 17 g of corn starch was used.

Example 4

Preparation of powders

The ingredients according to the following recipe were weighed and blended to provide the composition of the invention in powdery form.

Crude soya isoflavone powder	4.1 g
Galactosylsucrose (55% content)	10.0 g
Streptococcus E-23-17 lyophilizate	1.0 g
Vitamins & minerals	q.s.
Flavor	q.s.

Total	20 g

The Streptococcus E-23-17 lyophilizate was prepared by growing Streptococcus E-23-17 (FERM BP-6436) in a suitable liquid growth medium (GAM broth) (37 °C, 24-48 hours) and lyophilizing the resulting culture. The bacterial cell content of this freeze-dried powder was 10^9 -10^{10} cells/g.

Example 5

Preparation of granules

The ingredients according to the following recipe were weighed and blended to provide the composition of the invention in granular form.

Crude soya isoflavone powder	4.1 g
Galactosylsucrose (55% content)	10.0 g
Streptococcus E-23-17 lyophilizate	1.0 g
Sorbitol	q.s.
Vitamins & minerals	q.s.
Flavor	q.s.
Total	20 g

As the Streptococcus E-23-17 lyophilizate, the same freeze-dried powder as in Example 4 was used.

Example 6

(Microbial production of equol)

Using a water-soluble soya isoflavone material ("Fujiflavone P10", Fujicco) as the substrate, 1 ml of a

suspension of 10^7-10^9 cells of Streptococcus A6G-225 (FERM BP-6437) in GAM for culture of anaerobic bacteria was added to a 2.2% aqueous solution of the above substrate. The mixture was incubated aerobically at 37°C under stationary conditions for 96 hours and the amount of equol produced in the fermentation broth was measured by HPLC. The concentration of daidzin in the above aqueous solution was 1.083 mg/ml, and the concentration of daidzein was 0.014 mg/ml.

As a result, whereas no equol could be detected in the water-soluble soya isoflavone material, the equol content of the fermentation broth after 96 hours of culture was 613.0±8.7 μg/ml (means of 5 determinations ± S.D.). Neither daidzin nor daidzein was detected in the fermentation broth.

Using a substrate solution containing 0.01 mg/ml of daidzein (manufactured by Funakoshi, purity ≧99%) (5 mg of daidzein suspended in 2 ml of special grade methanol and diluted to 50 ml with BHI (brain heart infusion) medium) in lieu of the above water-soluble soya isoflavone material, equol was produced in otherwise the same manner as above. As a result, the amount of equol in the fermentation broth after 96 hours of culture was 17.9±1.4 μg/ml (mean of 5 determinations ± S.D.).

It is, therefore, clear that by utilizing the

microorganism of the invention, equol can be produced from daidzein with good efficiency.

Example 7

Preparation of a drink

Equol-containing fermentation broth	1.55 g
Glucose	5.00 g
Citric acid	0.5 g
Vitamins & minerals	q.s.
Flavor	q.s.
Water	q.s.
Total	200 ml

In the same manner as Example 6, 1 ml of a suspension of 10^7-10^9 cells of Streptococcus A6G-225 (FERM BP-6437) in GAM for culture of anaerobic bacteria was added to a 2.2% aqueous solution of water-soluble soya isoflavone material ("Fujiflavone P10", Fujicco) and the mixture was incubated aerobically at 37°C under stationary conditions for 96 hours. Using the equol-containing fermentation broth thus obtained and other ingredient according to the above recipe, the composition of the invention in the form of a drink was prepared.

Example 8

Preparation of a bar

Equol-containing fermentation broth	1.55 g
Butter	20.0 g

Sugar	20.0 g
Salt	Small amount
Egg	1/2
Wheat flour	80.0 g
Vitamins & minerals	q.s.
Flavor	q.s.
Milk	30.0 g

Using the equol-contain ing fermentation broth described in Example 7 in accordance with the above recipe, a dough was prepared, molded into a suitable bar form, and baked in an oven at 170°C for 15 minutes to provide a cake bar.

Example 9

Preparation of a jelly

Equol-containing fermentation broth	1.55 g
Fruit juice	50.0 g
Sugar	50.0 g
Agar	2.5 g
Vitamins & minerals	q.s.
Flavor	q.s.

Using the equol-containing fermentation broth described in Example 7 in accordance with the above recipe, the respective ingredients were heated up to 90 °C with constant stirring to dissolve the agar and the whole amount was poured into a suitable cup and cooled for gelation at

5-10°C to provide the composition of the invention in the form of a jelly.

INDUSTRIAL APPLICABILITY

The composition of the invention, when caused to be ingested or administered in the form of a food or a pharmaceutical product, proves useful for the prevention and alleviation of unidentified clinical syndrome or menopausal syndrome in middle-aged to elderly women.

CLAIMS

1. A composition comprising a daidzein-containing substance and a strain of microorganism capable of metabolizing daidzein to equol as essential ingredients.

2. The composition according to Claim 1 wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of Bacteroides ovatus, Streptococcus intermedius and Streptococcus constellatus.

3. The composition according to Claim 1 wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of Bacteroides E-23-15, which has been deposited as FERM BP-6435, Streptococcus E-23-17, which has been deposited as FERM BP-6436, and Streptococcus A6G-225, which has been deposited as FERM BP-6437.

4. The composition according to Claim 1 which further contains at least one component that favors the maintenance and growth of the strain of microorganism capable of metabolizing daidzein to equol.

5. The composition according to Claim 4 wherein the component that favors the maintenance and growth of the strain of microorganism capable of metabolizing daidzein to equol is at least one substance selected from the group

consisting of galactosylsucrose, soybean-oligosaccharide, lactulose, lactitol and fructo-oligosaccharide.

6. The composition according to Claim 1 wherein the daidzein-containing substance further contains at least one member selected from the group consisting of genistein, daidzin and genistin.

7. The composition according to Claim 1 wherein the daidzein-containing substance is soya isoflavone.

8. The composition according to Claim 1 for the prevention and treatment of unidentified clinical syndrome in middle-aged to elderly women, inclusive of menopausal syndrome.

9. The composition according to Claim 8 which is in a food form.

10. The composition according to Claim 9 wherein the food form is selected from the group consisting of drinks, dairy products, fermented milk, bars, granules, powders, capsules and tablets.

11. The composition according to Claim 8 which is a pharmaceutical dosage form.

12. The composition according to Claim 11 wherein the pharmaceutical dosage form is selected from the group consisting of aqueous solutions, emulsions, granules, powders, capsules and tablets.

13. A method for prevention and treatment of

unidentified clinical syndrome or menopausal syndrome in middle-aged to elderly women which comprises administering an effective amount of the composition according to claim 1 to a middle-aged or elderly woman who needs said prevention and treatment.

14. The use of a microorganism capable of utilizing a daidzein-containing substance or daidzein to elaborate equol for the production of a composition which is effective for the prevention and treatment of unidentified clinical syndrome or menopausal syndrome in middle-aged to elderly women.

15. A composition comprising equol which is obtained by causing a strain of microorganism capable of metabolizing daidzein to equol to act upon a daidzein-containing substance.

16. The composition according to Claim 15 wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of Bacteroides ovatus, Streptococcus intermedius and Streptococcus constellatus.

17. The composition according to Claim 15 wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of Bacteroides E-23-15, which has been deposited as FERM BP-6435, Streptococcus E-23-17, which

has been deposited as FERM BP-6436, and Streptococcus A6G-225, which has been deposited as FERM BP-6437.

18. The composition according to Claim 15 which further contains at least one component that favors the maintenance and growth of the strain of microorganism capable of metabolizing daidzein to equol.

19. The composition according to Claim 15 wherein the component that favors the maintenance and growth of the strain of microorganism capable of metabolizing daidzein to equol is at least one substance selected from the group consisting of galactosylsucrose, soybean-oligosaccharide, lactulose, lactitol and fructo-oligosaccharide.

20. The composition according to Claim 15 wherein said daidzein-containing substance further contains at least one member selected from the group consisting of genistein, daidzin and genistin.

21. The composition according to Claim 15 wherein the daidzein-containing substance is soya isoflavone.

22. The composition according to Claim 15 for the prevention and treatment of unidentified clinical syndrome in middle-aged to elderly women, inclusive of menopausal syndrome.

23. The composition according to Claim 22 which is in a food form.

24. The composition according to Claim 23 wherein the food form is selected from the group consisting of drinks, dairy products, fermented milk, bars, granules, powders, capsules and tablets.

25. The composition according to Claim 22 which is a pharmaceutical dosage form.

26. The composition according to Claim 25 wherein the pharmaceutical dosage form is selected from the group consisting of aqueous solutions, emulsions, granules, powders, capsules and tablets.

27. A method for prevention and treatment of unidentified clinical syndrome or menopausal syndrome in middle-aged to elderly women which comprises administering an effective amount of the composition according to claim 15 to a middle-aged or elderly woman who needs said prevention and treatment.

28. The use of a microorganism capable of utilizing a daidzein-containing substance or daidzein to elaborate equol for the production of a composition which is effective for the prevention and treatment of unidentified clinical syndrome or menopausal syndrome in middle-aged to elderly women.

29. A method of producing equol which comprises causing a strain of microorganism capable of metabolizing daidzein to equol to act upon daidzein.

30. The method according to claim 29 wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of Bacteroides ovatus, Streptococcus intermedius and Streptococcus constellatus.

31. The method according to Claim 29 wherein the strain of microorganism capable of metabolizing daidzein to equol is at least one member selected from the group consisting of Bacteroides E-23-15, which has been deposited as FERM BP-6435, Streptococcus E-23-17, which has been deposited as FERM BP-6436, and Streptococcus A6G-225, which has been deposited as FERM BP-6437.

32. A strain of microorganism selected from the group consisting of Bacteroides E-23-15, which has been deposited as FERM BP-6435, Streptococcus E-23-17, which has been deposited as FERM BP-6436, and Streptococcus A6G-225, which has been deposited as FERM BP-6437.

ABSTRACT

This invention provides a composition comprising a daidzein-containing substrate and a strain of micro-organism capable of metabolizing daidzein to equol as essential ingredients. This composition is effective in the prevention and alleviation of unidentified clinical syndrome inclusive of menopausal syndrome in middle-aged to elderly women for which no effective means of prevention or alleviation has heretofore been avai lable.

Declaration and Power of Attorney for Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

As a below named inventor, I hereby declare that:

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

My residence, post office address and citizenship are as stated next to my name,

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者(下記の氏名が一つの場合)もしくは最初かつ共同発明者であると(下記の名称が複数の場合)信じています。

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

_____

_____

_____

ISOFLAVONE-CONTAINING COMPOSITION

_____

_____

上記発明の明細書(下記の欄でX印がついていない場合は、本書に添付)は、

the specification of which is attached hereto unless the following box is checked:

☐ ___月___日に提出され、米国出願番号または特許協定条約

国際出願番号を _____ とし、

(該当する場合) _____ に訂正されました。

☒ was filed on August 4, 1998
as United States Application Number or
PCT International Application Number

PCT/JP98/03460 and was amended on

_____ (if applicable).

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

私は、連邦規則法典第37編第1条56項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

Japanese Language Declaration
(日本語宣言書)

私は、米国法典第35編第119条(a)-(d)項又は第365条(b)項に基き下記の、米国以外の国の少なくとも一カ国を指定している特許協力条約第365条(a)項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

I hereby claim foreign priority under Title 35, United States Code, Section 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Applications
外国での先行出願

(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	Priority Not Claimed 優先権主張なし
214604/1997	Japan	08/08/1997	☐
			☐
			☐

私は、第35編米国法典119条(e)項に基づいて下記の米国特許出願規定に記載された権利をここに主張致します。

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)

私は、下記の米国法典第35編第120条に基づいて下記の米国特許出願に記載された権利、又は米国を指定している特許協力条約第365条(c)に基づく権利をここに主張します。又、本出願の各請求範囲の内容が米国法典第35編第112条第1項又は特許協力条約で規定された方法で先行する米国特許出願に開示されていない限り、その先行米国出願書提出日以降で本出願書の日本国内又は特許協力条約国際出願提出日までの期間中に入手された、連邦規則法典第37編第1条第56項で定義された特許資格の有無に関する重要な情報について開示義務があることを認識しています。

I hereby claim the benefit of Title 35, United States Code Section 120 of any United States application(s), or 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose any material information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況: 特許許可済、係属中、放棄済)

私は、私自身の知識に基づいて本宣言中で私が行う表明が真実であり、かつ私の入手した情報と私の信ずるところに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行えば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Japanese Language Declaration

（日本語宣言書）

委任状：私は、下記の発明者として、本出願に関する一切の手続きを米国特許商標局に対して遂行する弁理士又は代理人として、下記のものを指名致します。（弁護士、又は代理人の氏名及び登録番号を明記のこと）

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith *(list name and registration number)*

29

John H. Mion, Reg. No. 18,879; Thomas J. Macpeak, Reg. No. 19,292; Robert J. Seas, Jr., Reg. No. 21,092; Darryl Mexic, Reg. No. 23,063; Robert V. Sloan, Reg. No. 22,775; Peter D. Olexy, Reg. No. 24,513; J. Frank Osha, Reg. No. 24,625; Waddell A. Biggart, Reg. No. 24,861; Louis Gubinsky, Reg. No. 24,835; Neil B. Siegel, Reg. No. 25,200; David J. Cushing, Reg. No. 28,703; John R. Inge, Reg. No. 26,916; Joseph J. Ruch, Jr., Reg. No. 26,577; Sheldon I. Landsman, Reg. No. 25,430; Richard C. Turner, Reg. No. 29,710; Howard L. Bernstein, Reg. No. 25,665; Alan J. Kasper, Reg. No. 25,426; Kenneth J. Burchfiel, Reg. No. 31,333; Gordon Kit, Reg. No. 30,764; Susan J. Mack, Reg. No. 30,951; Frank L. Bernstein, Reg. No. 31,484; Mark Boland, Reg. No. 32,197; William H. Mandir, Reg. No. 32,156; Scott M. Daniels, Reg. No. 32,562; Brian W. Hannon, Reg. No. 32,778; Abraham J. Rosner, Reg. No. 33,276; Bruce E. Kramer, Reg. No. 33,725; Paul F. Neils, Reg. No. 33,102 and Brett S. Sylvester, Reg. No. 32,765

書類送付先：

Send Correspondence to:

SUGHRUE, MION, ZINN, MACPEAK & SEAS, PLLC
2100 Pennsylvania Avenue, N.W., Washington, D.C. 20037-3202

直通電話連絡先：（名称及び電話番号）

Direct Telephone Calls to: ***(name and telephone number)***

(202)293-7060

唯一又は第一発明者名	Full name of sole or first inventor UCHIYAMA Shigeto
発明者の署名　日付	Inventor's signature　Date Shigeto Uchiyama　Jan. 14, 2000
住所	Residence Miyaki-gun, Saga 841-0204, Japan
国籍	Citizenship Japan　JPX
郵便の宛先	Post office address 1157-1, Oaza Miyaura, Kiyamamachi,
	Miyaki-gun, Saga 841-0204, Japan
第二共同発明者名（該当する場合）	Full name of second joint inventor, if any UENO Tomomi
第二発明者の署名　日付	Second inventor's signature　Date Tomomi Ueno　Jan. 14, 2000
住所	Residence Kurume-shi, Fukuoka 830-0027, Japan
国籍	Citizenship Japan　JPX
郵便の宛先	Post office address Daiichihaimato Shigyo 302, 5-8-14,
	Nagatoishi, Kurume-shi, Fukuoka 830-0027, Japan

（第三以降の共同発明者についても同様に記載し、署名をすること。）

(Supply similar information and signature for third and subsequent joint inventors.)

Japanese Language Declaration
(日本語宣言書)

第三共同発明者名(該当する場合)	Full name of third joint inventor, if any KUMEMURA Megumi
第三発明者の署名 日付	Third inventor's signature Date Megumi Kumemura Jan. 14, 2000
住所	Residence Kurume-shi, Fukuoka 839-0862, Japan
国籍	Citizenship Japan
郵便の宛先	Post office address Famiru Haitsu Kurume 608, 1175-1,
	Nonakamachi, Kurume-shi, Fukuoka 839-0862, Japan
第四共同発明者名(該当する場合)	Full name of fourth joint inventor, if any IMAIZUMI Kiyoko
第四発明者の署名 日付	Fourth inventor's signature Date Imaizumi Kiyoko Jan. 14, 2000
住所	Residence Kurume-shi, Fukuoka 830-0027, Japan
国籍	Citizenship Japan
郵便の宛先	Post office address Daiichihaimato Shigyo 302, 5-8-14,
	Nagatoishi, Kurume-shi, Fukuoka 830-0027, Japan
第五共同発明者名(該当する場合)	Full name of fifth joint inventor, if any MASAKI Kyosuke
第五発明者の署名 日付	Fifth inventor's signature Date Kyosuke Masaki Jan. 14, 2000
住所	Residence Kurume-shi, Fukuoka 830-0027, Japan
国籍	Citizenship Japan
郵便の宛先	Post office address 2-2-80-901, Nagatoishi, Kurume-shi,
	Fukuoka 830-0027, Japan
第六共同発明者名(該当する場合)	Full name of sixth joint inventor, if any SHIMIZU Seiichi
第六発明者の署名 日付	Sixth inventor's signature Date Seiichi Shimizu Jan. 14, 2000
住所	Residence Tosu-shi, Saga 841-0051, Japan
国籍	Citizenship Japan
郵便の宛先	Post office address 1001, 1237-2, Motomachi, Tosu-shi, Saga
	841-0051, Japan